Rev Bras Farmacogn 24(2014): 67-72

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original article

Pharmacological evaluation of bee venom and melittin

Camila G. Dantas[a], *Tássia L.G.M. Nunes*[a], *Tâmara L.G.M. Nunes*[a], *Ailma O. da Paixão*[a], *Francisco P. Reis*[a], *Waldecy de L. Júnior*[b], *Juliana C. Cardoso*[a], *Kátia P. Gramacho, Margarete Z. Gomes* [a,*]

[a]*Laboratório de Morfologia e Biologia Estrutural, Instituto de Tecnologia e Pesquisa, Universidade Tiradentes, Aracaju, SE, Brazil.*
[b]*Departamento de Morfologia, Universidade Federal de Sergipe, Aracaju, SE, Brazil*

ARTICLE INFO

Article history:
Received 13 November 2013
Accepted 12 February 2014

Keywords:
Bee venom
Dopamine
Haloperidol

ABSTRACT

The objective of this study was to identify the pharmacological effects of bee venom and its major component, melittin, on the nervous system of mice. For the pharmacological analysis, mice were treated once with saline, 0.1 or 1.2 mg/kg of bee venom and 0.1 mg/kg of melittin, subcutaneously, 30 min before being submitted to behavioral tests: locomotor activity and grooming (open-field), catalepsy, anxiety (elevated plus-maze), depression (forced swimming test) and apomorphine-induced stereotypy. Haloperidol, imipramine and diazepam were administered alone (positive control) or as a pre-treatment (haloperidol). The bee venom reduced motor activity and promoted cataleptic effect, in a similar manner to haloperidol. These effects were decreased by the pretreatment with haloperidol. Both melittin and bee venom decreased the apomorphine-induced stereotypies. The data indicated the antipsychotic activity of bee venom and melittin in a murine model.

Introduction

The bee venom (BV) produced by Africanized honey bee-AHB (*Apis mellifera* L.) is a complex mixture of enzymes, lipids, amino acids, carbohydrates and peptides like apamin and melittin. It also contains dopamine and phospholipase A_2 (Hider, 1988; Sciani et al., 2010; Ferreira-Junior et al., 2010). Melittin constitutes 40 to 60% of dry whole honeybee venom, and this peptide has various biological activities, including high anti-inflammatory activity (Habermann, 1972; Gauldie et al., 1976; Son et al., 2007).

Experimental studies have shown neuroprotective effects of BV and melittin in amyotrophic lateral sclerosis (Yang et al., 2010; 2011) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease *in vivo* (Doo et al., 2010; Kim et al., 2011a; Chung et al., 2012; Doo et al., 2012; Cho et al., 2012) and against glutamatergic excitotoxicity in neuronal and glial cell cultures (Lee et al., 2012). Effectiveness of BV acupuncture was also demonstrated for idiopathic Parkinson's disease (Cho et al., 2012).

The subcutaneous administration of BV has also been shown to induce the activation of catecholaminergic neurons in the arcuate nucleus in the hypothalamus of rats (Kwon et al., 2004) and in dopaminergic nuclei, including the

* Corresponding author.
E-mail: margarete_zanardo@itp.org.br; margarete_zanardo@unit.br (M.Z. Gomes).
0102-695X/$ - see front matter © 2014 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.
DOI: 10.1590/0102-695X20142413365

nucleus accumbens, substantia nigra, and locus coeruleus. When combined with acupuncture, it was able to reduce methamphetamine-induced hyperactivity (Kim et al., 2011b). In addition, it has been shown that BV exhibited no signs of toxicity when administered within a therapeutic range subcutaneously (*s.c.*) (Kim et al., 2004).

Despite the wide range of proposed uses for BV and melittin, the pharmacological effects in the central nervous system that result from their administration under physiological conditions have been poorly addressed. The development of new therapeutic approaches to mental disorders and neurodegenerative diseases remains a challenge. So, this study aimed to examine the pharmacological effects of BV and melittin in mice, with particular emphasis on dopaminergic related behaviors.

Materials and methods

Animals and ethical statements

Male Swiss albino mice (25-30 g) were maintained in a temperature and light cycle-controlled environment with free access to water and food. All procedures were in accordance with the standards of the Brazilian College of Animal Experimentation, and the experimental protocol was approved by the Ethical Committee for Animal Use of the Tiradentes University, Aracaju-SE, Brazil (approval number 010213).

Drugs

The bee venom (BV) was purchased from an apiary in the city of São Roque-SP, in the Brazilian Southeast region, it was obtained by electric extraction. Other commercial chemicals were used: melittin (Sigma®, São Paulo, Brazil), haloperidol (Janssen-Cilag Pharmaceuticals® Ltda, São Paulo, Brazil), Imipramine hydrochloride (Novartis Biosciences® S.A., São Paulo, Brazil) and diazepam (Union SA National Pharmaceutical Chemistry®, São Paulo, Brazil).

Assays

Pharmacological evaluation of the acute effects

The animals received vehicle, melittin (0.1 mg/kg) or BV (0.1 mg/kg or 1.2 mg/kg). Both BV and melittin were diluted in saline [0.9%, 40 µl], administered subcutaneously (*s.c.*); the doses were chosen based on previous articles (Doo et al., 2010; Kim et al., 2011abc; Lee et al., 2001; Yang et al., 2010; 2011). Behavioral tests were performed 30 min after the treatments. The data were compared with the obtained after treatment with the antipsychotic haloperidol [0.2 mg/kg, intraperitoneal (*i.p.*)], imipramine (10 mg/kg. *i.p.*) and diazepam (1 mg/kg. *i.p.*). In the groups that received haloperidol and BV, haloperidol was injected 30 min prior the BV.

Catalepsy test

Catalepsy, defined as failure to correct an externally imposed posture, was evaluated according to the standard horizontal bar hanging test by placing the animals with both forelegs over a horizontal glass bar with 0.5 cm diameter, and 4.5 cm high from the ground (Sanberg et al., 1988). The time during which the mouse maintained this position was recorded for up to 300 s, with three tries allowed to replace the animal in the cataleptic position. Catalepsy was considered to have ended when the forepaw touched the floor or when the mouse climbed the bar (Del Bel et al., 2010).

The open-field test

To evaluate the motor and emotional state, an open-field test was performed (Whimbey and Denenberg, 1967; Prut and Belzung, 2003). The following parameters were evaluated for 5 min: locomotion or crossings (number of line crosses), rearings (the number of times the mouse stands on its hind legs), grooming (the number of times the mouse "washes" itself by licking during the observation period) and time spent in the central area. The equipment was made of white colored wood, and it consisted of a quadrilateral with a rectangular area of 4830.25 cm^2 and walls 34.5 cm high, with the base subdivided into sixteen quadrants (Hongxing et al., 2007; O'Leary et al., 2013).

Apomorphine-induced stereotipy

The apomorphine-induced stereotypies were measured by a score scale (from 0 to 6 where 0 = asleep or stationary; 1 = active; 2 = predominantly active but with bursts of stereotyped sniffing and rearing; 3 = constant stereotyped activity such as sniffing, rearing or head bobbing, but with locomotor activity still present; 4 = constant stereotyped activity maintained in one location; 5 = constant stereotyped activity but with bursts of licking and/or gnawing and biting; and 6 = continual licking and or gnawing of cage grids) after the application of apomorphine (20 mg/kg, *s.c.*, Sigma-Aldrich Brasil Ltda., São Paulo, Brazil). Each animal was observed for 10 s intervals every 10 min for 60 min (Setler et al., 1976). The results were expressed as the mean sum of scores that were obtained for each group.

The elevated plus-maze test

To evaluate possible action on anxiety, the elevated plus-maze was used. The number of entries and the stay duration of the animals in the open and closed arms (Pellow et al., 1985) were analyzed in an apparatus with a base 45 cm (height) from the ground, with two open arms (50 × 10 cm) and two enclosed arms of the same size, with walls 40 cm in height uncovered on the top. Each mouse was observed for 5 min.

The forced swimming test

The forced swimming test was carried out as previously described (Porsolt et al., 1977) in order to evaluate a possible antidepressant effect. The test consisted of two swim sessions separated by a period of 24 h. In the first session, the animals were placed in a circular water tank (30 cm high) for 15 min. After a period of intense movement, the animals acquire a posture of immobility, with minimal movements to keep the head out of the water. In the second session (test), the animals underwent the same procedure for 5 min. the swimming and immobility times were evaluated.

Statistical analysis

After applying the Kolmogorov-Smirnov normality test, the gaussian data (mean ± standard error of the mean) of the catalepsy, open-field, elevated plus-maze and forced swimming tests were subjected to a one-way analysis of variance (ANOVA) followed by a Tukey post hoc test. Non Gaussian data (scores) from apomorphine-induced stereotypy were analyzed by Kruskal-Wallis test followed by a Dunn's multiple comparison test. All statistical analysis was performed using SPSS Statistics 19.0 software. Values of $p < 0.05$ were considered statistically significant.

Results

Pharmacological evaluation of the acute effects

Cataleptic effect

The statistical analysis of catalepsy revealed a significant difference between treatments ($F_{5-59} = 8.086$, $p < 0.0001$; ANOVA followed by Tukey post-test), as illustrated in Table 1. The groups treated with 0.1 mg/kg of BV and melittin (0.1 mg/kg) recorded lesser time spent on the bar. In contrast, the administration of 1.2 mg/kg of BV induced cataleptic activity, an effect similar to that observed with haloperidol treatment. When the treatments were combined, it was observed that a lower dose of BV did not modify the action of haloperidol, while the administration of 1.2 mg/kg reversed the observed effects. The animals that received saline solution did not remain on the bar and thus were not subject to statistical analysis.

Table 1
Effect of bee venom and melittin administration on the catalepsy test.

Group	Treatment	*n*	Dose (mg/kg)	Catalepsy (s)
1	Saline + Saline	5	-	-
2	Saline + Melittin	6	0.1	1.9 ± 0.9
3	Saline + BV	5	0.1	2.5 ± 0.6
4	Saline + BV	6	1.2	87.2 ± 20.6[b]
5	Haloperidol + Saline	4	0.2	75.7 ± 28.8[a]
6	Haloperidol + BV	4	0.2 and 0.1	92.6 ± 16.7[b]
7	Haloperidol + BV	4	0.2 and 1.2	3.3 ± 0.6

The results are expressed as mean ± standard error of the mean (SEM).
[a]$p < 0.05$ from groups 2; 3 and 7.
[b]$p < 0.01$ from groups 2; 3 and 7 [one-way analysis of variance (ANOVA) followed by Tukey post-test].
BV, bee venom, *n*, number of animals per group. The animals that receive saline solution did not remain on the bar and thus no statistical analysis was performed.

Locomotor and exploratory activity

In the open-field test, the administration of BV at a dose of 1.2 mg/kg caused a significant reduction in the number of crossings compared to the other treatments. The same result was observed after the administration of haloperidol. This effect was not observed when the treatments were associated (Table 2, ANOVA followed by Tukey post-test, $F_{6-34} = 14.122$, $p < 0.0001$). When rearing was examined, both doses of bee venom and treatment with haloperidol resulted in a significant reduction compared to that seen in controls ($F_{6-34} = 8.687$, $p < 0.0001$, ANOVA followed by Tukey post-test). The reduction remained significant when the lowest dose of BV was preceded by haloperidol, but the effect was reversed in the group receiving 1.2 mg/kg of BV (Table 2). In addition, there was a significant decrease in grooming behavior after 1.2 mg/kg of BV compared to the saline group ($F_{6-34} = 3.525$, $p = 0.010$, ANOVA, followed by Tukey post-test).

Table 2
Effects of the bee venom and melittin on the open-field test.

Group	Treatment	*n*	Dose (mg/kg)	Crossing (5 min)	Rearing (5 min)	Grooming (5 min)
1	Saline	8	----	164.0 ± 12.7	32.6 ± 4.4	2.4 ± 0.3
2	Melittin	6	0.1	161.2 ± 12.0	34.3 ± 2.1	1.1 ± 0.27
3	BV	5	0.1	139.8 ± 11.6	7.6 ± 2.4[b]	1.2 ± 0.5
4	BV	6	1.2	44.8 ± 14.7[c]	7.8 ± 4.8[b]	0.8 ± 0.31[a]
5	Haloperidol	4	0.2	65.0 ± 22.6[b]	9.0 ± 3.6[b]	1.2 ± 0.2
6	Haloperidol + BV	4	0.2 and 0.1	140.7 ± 10.2	6.0 ± 5.0[b]	1.5 ± 0.3
7	Haloperidol + BV	5	0.2 and 1.2	110.0 ± 20.2	27.8 ± 15.5	1.2 ± 0.2

The results are expressed as mean ± SEM.
[a]$p < 0.05$ from group 1.
[b]$p < 0.01$ from groups 1; 2; 3; 6 (only in the groups unmarked with similar letter) and $p < 0.05$ from group 7.
[c]$p < 0.001$ from groups 1; 2; 3; 6 and $p < 0.05$ from group 7 [one-way analysis of variance (ANOVA) followed by Tukey post-test].
BV, bee venom, *n* = number of animals per group.

Neuroleptic effect

Both doses of BV and melittin decreased apomorphine-induced stereotypies ($p = 0.006$, Kruskal-Wallis test followed by Dunn's multiple comparison test). However, there were no dose-dependent effects ($p = 0.82$). The results are shown in the Figure 1.

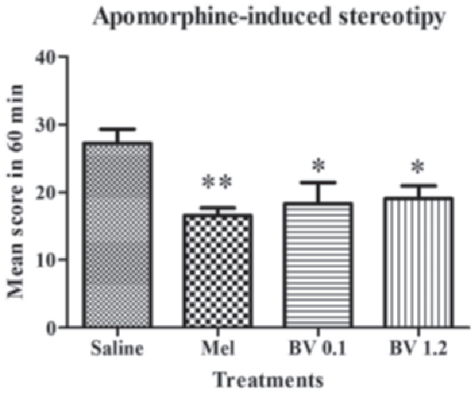

Figure 1 - The treatment with bee venom [(BV), 0.1 mg/kg and 1.2 mg/kg, and melittin (0.1 mg/kg) decreased apomorphine-induced stereotyped behaviors in mice. BV, melittin or saline (40 µl) were administered 30 min before apomorphine (20 mg/kg, *s.c.*), and the measurements were taken for 10 s in intervals of 10 min for 60 min. The results are expressed as mean ± SEM of 10 s counts. $^{*}p < 0.05$ and $^{**}p < 0.01$ from saline group (Kruskal-Wallis test followed by Dunn's multiple comparison test, n = 8-12).

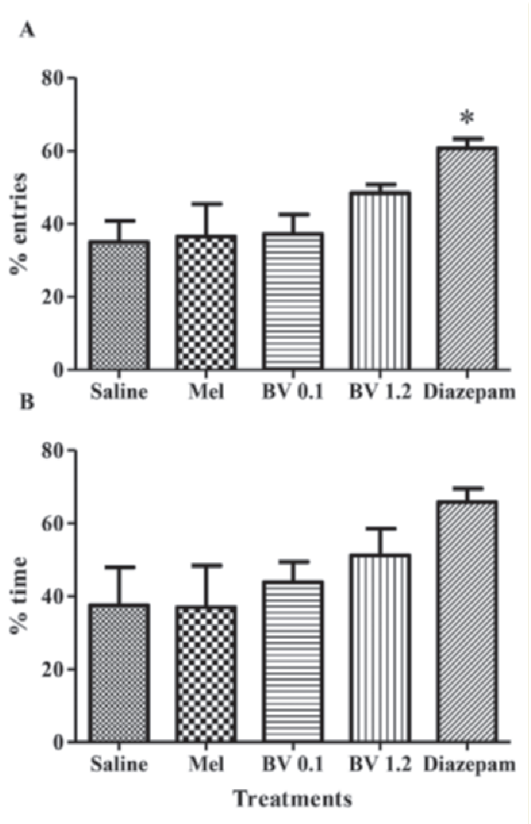

Figure 2 - Effect of the BV and melittin on the elevated plus-maze test. Mice received saline (40 µl), bee venom (BV) or melittin subcutaneous (*s.c.*) and diazepam (1 mg/kg, *i.p.*) 30 min before the test. The percentage of entries (**A**) and time spent (**B**) in open arms are registered. Data are expressed as mean ± SEM.
$^{*}p < 0.05$ compared to other groups (one way ANOVA followed by Tukey post-test, n = 6-9).

Anxiolytic effect

Regarding to the elevated plus-maze, the acute treatment with bee venom and melittin did not result in a significant effect on the assessment of anxiolytic activity. No changes were observed on the time spent in the open arms between the groups ($F_{4-31} = 2.196$, $p = 0.096$). Only the administration of diazepam significantly increased the number of entries into the open arms ($F_{4-31} = 3.598$, $p = 0.018$, one way ANOVA followed by Tukey post-test, Figure 2).

Antidepressant effect

Both doses of BV induced a significant increase in the immobility time observed in the forced swimming test, compared to imipramine ($F_{4-30} = 3.219$, $p = 0.028$, one way ANOVA followed by Tukey post-test, Table 3). From saline group, BV induced about 70% increase in the immobility time, and melittin induced about 20%, although these differences were not found statically relevant.

Table 3
Effect of the bee venom and melittin on the forced swimming test.

Group	Treatment	*n*	Dose (mg/kg)	Immobility time (s)
1	Saline	7	-	55.1 ± 25.7
2	Melittin	5	0.1	64.8 ± 14.3
3	BV	6	0.1	95.7 ± 15.7[a]
4	BV	6	1.2	99.5 ± 15.4[a]
5	Imipramine	8	10	27.4 ± 9.7

The results are expressed as mean ± SEM.
[a]$p < 0.05$, bee venom (BV) groups compared to imipramine [one-way analysis of variance (ANOVA) followed by Tukey post-test].

Discussion

The results of this study showed that BV induced catalepsy in rodents, decreased apomorphine-induced stereotypies, and changed the parameters associated with motor activity in the open-field test. These effects were similar to the actions that described for the neuroleptic haloperidol, and a significant interaction was found when the bee venom treatment was combined with haloperidol.

The haloperidol-induced catalepsy is characterized by the occupation of striatal dopamine receptors (Sanberg, 1980). The finding of a cataleptic effect reduction associated to the treatments might suggest the occupation of these receptors by BV, and a competitive interaction of BV with haloperidol. The components that are present in BV have been related to dopaminergic transmission. Studies revealed that melittin decreases the uptake of dopamine by acting directly on the dopamine transporter, and inhibits the binding of D2 receptors with antagonist in cell culture (Keith et al., 2011; 2012). The other bioactive compound in BV, apamin, acts as a blocker of

potassium channels and may cause an increased activity of dopaminergic neurons through D2 receptors (Vandecasteele et al., 2011). The microinjection of apamin in the nucleus accumbens of rats has been shown to produce increased motor activity and augmented brain levels of dopamine and its metabolites (Steketee and Kalivas, 1990). The behavioral effects were distinct to those observed in the open-field test in this study, nevertheless, it is noteworthy that the authors also observed a reversal by pre-treatment with haloperidol. Those findings are consistent with this study's results that revealed that the administration of bee venom and haloperidol resulted in the reversal of the cataleptic effects, indicating that the active ingredients of bee venom may mediate the catalepsy that could be induced by D2 receptor occupancy.

Since the modifications in the spontaneous motor activity may be mediated by D2 receptors (Zuo et al., 2008) while D1 receptors are involved in grooming (Drago et al., 1999), the results point to an interaction of BV with the dopaminergic system. In agreement with this hypothesis, we observed a reduction in stereotypies induced by apomorphine after acute bee venom and melittin treatment. The stereotypy results from apomorphine interactions with dopamine receptors (Randrup and Munkvad, 1974) and it is a test frequently used for evaluating the properties of antipsychotic drugs.

To date, previous studies in the literature have investigated the effects of BV on behaviors related to pain and inflammation (Roh et al., 2006; Merlo et al., 2011), the prevention of amphetamine addiction (Kwon et al., 2010), and neuroprotective effects against neuronal death (Kim et al, 2011a). One study conducted (Kim et al., 2004) with lower doses reported no effects of the BV and its sub-fractions on sleep-induction time and duration, motor function in mice (rota-rod test), general spontaneous activity and pentyleneteterazole-induced convulsions in mice. An antipsychotic activity would be, therefore, a novel therapeutic effect of BV if lower doses are tested and proved efficient.

More experiments with selective antagonists of different dopamine receptors are needed to elucidate the mechanism of action of the BV and melittin, but it is important to note that melittin displayed action on the apomorphine-induced stereotypies without side effects on motor performance or catalepsy with the dose tested. Neither BV nor melittin promoted depressant effect as assessed by the forced swimming test. It has been well demonstrated that drugs with antidepressant activity reduce the time during the animals remained immobile (Borsini and Meli, 1988). Thus, the results indicate a neuroleptic-like but not antidepressant effect for BV.

In addition, there was no effect of the BV and melittin on the anxiety parameters assessed by the elevated plus-maze test. It can indicate an absence of interaction with GABAergic system. Diazepam has been employed in behavioral pharmacology as a reference compound for potential anxiolytic-like substances (Mizushige et al., 2013). Here, it increased the number of entries in the open arms as expected. Although diazepam promoted an increase about 30% on the percent of the time spent in the open arms from the saline group, the statistical analysis failed to reveal a significant effect. This may be due the high SEM in the control groups and/or the reduced number of animals in some groups (n = 5-9).

Conclusions

These results indicate an interaction of BV with the dopaminergic system that might benefit the development of new BV-based strategies to treat mental disorders. Also, its major component melittin showed antipsychotic properties without the classic side effects of neuroleptic-like drugs. More studies are needed to elucidate the mechanisms of action of these compounds and its potential for future clinical applications.

Authors' contributions

CGD contributed in running the laboratory work, analysis of the data and drafted the paper. CGD, TLGMN, TLGMN and AOP contributed to biological studies. FPR, WLJ and JCC contributed to critical reading of the manuscript. KPG and MZG designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts interest.

Acknowledgments

The authors thank the Fundação de Apoio a Pesquisa e à Inovação Tecnológica do Estado de Sergipe (FAPITEC) and CNPQ–Brazil, for their financial support.

REFERENCES

Borsini, F., Meli, A., 1988. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl) 94, 147-160.

Cho, S-Y., Shim, S-R., Rhee, H.Y., Park, H-J., Jung, W-S., Moon, S-K.M., Park, J-M., Ko, C-N., Cho, K-H., Park, S-U., 2012. Effectiveness of acupuncture and bee venom acupuncture in idiopathic Parkinson's disease. Parkinsonism Relat. D. 18, 948-952.

Chung, E.S., Kim, H., Lee, G., Park, S., Kim, H., Bae, H., 2012. Neuro-protective effects of bee venom by suppression of neuroinflammatory responses in a mouse model of Parkinson's disease: Role of regulatory T cells. Brain Behav. Immun. 26, 1322-1330.

Del-Bel, E.A., Guimarães, F.S., Joca, S.R., Echeverry, M.B., Ferreira, F.R., 2010. Tolerance to the cataleptic effect that follows repeated nitric oxide synthase inhibition may be related to functional enzymatic recovery. J. Psychopharmacol. 24, 397-405.

Doo, A.R., Kim, S.N., Kim, S.T., Park, J.Y., Chung, S.H., Choe, B.Y., Chae, Y., Lee, H., Yin, C.S., Park, H.J., 2012. Bee venom protects SH-SY5Y human neuroblastoma cells from 1-methyl-4-phenylpyridinium-induced apoptotic cell death. Brain Res. 1429, 106-115.

Doo, A.R., Kim, S.T., Kim, S.N., Moon, W., Yin, C.S., Chae, Y., Park, H.K., Lee, H., Park, H.J., 2010. Neuroprotective effects of bee venom pharmaceutical acupuncture in acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridineinduced mouse model of Parkinson's disease. Neurol. Res. 32, 88-91.

Drago, F., Contarino, A., Busà, L., 1999. The expression of neuropeptide-induced excessive grooming behavior in dopamine D1 and D2 receptor-deficient mice. Eur. J. Pharmacol. 365, 125-131.

Ferreira-Junior, R.S., Sciani, J.M., Marques-Porto, R., Junior, A.L., Orsi, R.O., Barraviera, B., Pimenta, D.C., 2010. Africanized honey bee (*Apis mellifera*) venom profiling: Seasonal variation of melittin and phospholipase A_2 levels. Toxicon 56, 355-362.

Gauldie. J,, Hanson, J.M., Rumjanek, F.D., Shipolini, R.A., Vernon, C.A., 1976. The peptide components of bee venom. Eur. J. Biochem. 61, 369-376.

Habermann, E., 1972. Bee and wasp venoms. Science 177, 314-322.

Hider, R.C., 1988. Honeybee venom: A rich source of pharmacologically active peptides. Endeavour 12, 60-65.

Hongxing, Z., Nancai, Y., Guofu, H., Jianbo, S., Yanxia, W., Hanju, H., Qian, L., Mei, M., Yandong, Y., Hao, Y., 2007. Neuroprotective effects of purslane herb aquenous extracts against D-galactose induced neurotoxicity. Chem. Biol. Interact. 170, 145-152.

Keith, D.J., Eshleman, A.J., Janowsky, A., 2011. Melittin stimulates fatty acid release through non-phospholipase-mediated mechanisms and interacts with the dopamine transporter and other membrane-spanning proteins. Eur. J. Pharmacol. 650, 501-510.

Keith, D.J., Wolfrum, K., Eshleman, A.J., Janowsky, A. 2012. Melittin initiates dopamine transporter internalization and recycling in transfected HEK-293 cells. Eur. J. Pharmacol. 690, 13-21.

Kim, H-W., Kwon, Y-B., Ham, T-W., Roh, D.H., Yoon, S-Y., Kang, S-Y., Yang, I-S., Han, H-J., Lee, H-J., Beitz, A.J., Lee, J.H., 2004. General pharmacological profiles of bee venom and its water soluble fractions in rodent models. J. Vet. Sci. 5, 309-318.

Kim, J.I., Yang, E.J., Lee, M.S., Kim, Y.S., Huh, Y., Cho, I.H., Kang, S., Koh, H.K., 2011a. Bee venom reduces neuroinflammation in the MPTP-induced model of Parkinson's disease. Int. J. Neurosci, 121, 209-217.

Kim, K.W., Kim, H.W., Li, J., Kwon, Y.B., 2011b. Effect of bee venom acupuncture on methamphetamine-induced hyperactivity, hyperthermia and Fos expression in mice. Brain Res. Bull. 84, 61-68.

Kim, S.J.; Park, J.H., Kim, K.H., Lee, W.R., Kim, K.S., Park, K.K., 2011c Melittin inhibits atherosclerosis in LPS/high-fat treated mice through atheroprotective actions. J. Atheroscler. Thromb. 18, 1117-1126.

Kwon, Y.B., Han, H.J., Beitz, A.J., Lee, J.H., 2004. Bee venom acupoint stimulation increases Fos expression in catecholaminergic neurons in the rat brain. Mol. Cells 17, 329-333.

Kwon, Y.B., Li, J., Kook, J.A., Kim, T.W., Jeong, Y.C., Filho, J.S., Lee, H., Kim, K.W., Lee, J.H., 2010. Bee venom suppresses methamphetamine-induced conditioned place preference in mice. Neurol. Res. 32, 101-106.

Lee, J.H., Kwon, Y.B., Han, H.J., Mar, W.C., Lee, H.J., Yang, I.S., Beitz, A.J., Kang, S.K., 2001. Bee venom pretreatment has both an antinociceptive and anti-inflammatory effect on carrageenan-induced inflammation. J. Vet. Med. Sci. 63, 251-259.

Lee, S.M., Yang, E.J., Choi, S.M., Kim, S.H., Baek, M.G., Jiang, J.H., 2012. Effects of bee venom on glutamate-induced toxicity in neuronal and glial cells. Evid. Based Complement. Alternat. Med. Article ID 368196, DOI: 10.1155/2012/368196.

Merlo, L.A., Bastos, L.F., Godin, A.M., Rocha, L.T., Nascimento, E.B.J.R., Paiva, A.L., Moraes-Santos, T., Zumpano, A.A., Bastos, E.M., Heneine, L.G., Coelho, M.M., 2011. Effects induced by *Apis mellifera* venom and its components in experimental models of nociceptive and inflammatory pain. Toxicon 57, 764-771.

Mizushige, T., Kanegawa, N., Yamada, A., Ota, A., Kanamoto, R., Ohinata, K., 2013. Aromatic aminoacid-leucine dipeptides exhibit anxiolytic-like activity in young mice. Neurosci. Lett. 543, 126-129.

O'Leary, T.P., Gunn, R.K., Brown, R.E., 2013. What are we measuring when we test strain differences in anxiety in mice? Behav. Genet. 43, 34-50.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Meth. 14, 149-167.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327-336.

Prut, L., Belzung, C., 2003. The open-field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pahrmacol. 463, 3-33.

Randrup, A., Munkvad, I., 1974. Pharmacology and physiology of stereotyped behavior. J. Psychiatr. Res. 11, 1-10.

Roh, D.H., Kim, H.W., Yoon, S.Y., Kang, S.Y., Kwon, Y.B., Cho, K.H., Han, H.J., Ryu, Y.H., Choi, S.M., Lee, H.J., Beitz, A.J., Lee, J.H., 2006. Bee venom injection significantly reduces nociceptive behavior in the mouse formalin test via capsaicin-insensitive afferents. J. Pain 7, 500-512.

Sanberg, P.R., Bunsey, M.D., Giordano, M., Norman, A.B., 1988. The catalepsy test: its ups and downs. Behav. Neurosci. 102, 748-759.

Sanberg, P.R., 1980. Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature 284, 472-473.

Sciani, J.M., Marques-Porto, R., Lourenço Junior, A., Orsi, R. O., Ferreira Junior, R.S., Barraviera, B., Pimenta, D.C., 2010. Identification of a novel melittin isoform from Africanized Apis mellifera venom. Peptides 31, 1473-1479.

Setler, P., Sarau, H., McKenzie, G., 1976. Differential attenuation of some effects of haloperidol in rats given scopolamine. Eur. J. Pharmacol. 39, 117-126.

Son, D.J., Lee, J.W., Lee, Y.H., Song, H.S., Lee, C.K., Hong, J.T., 2007. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol Ther 115, 246-270.

Steketee, J.D., Kalivas, P.W., 1990. Effect of microinjections of apamin into the A10 dopamine region of rats: a behavioral and neurochemical analysis. J. Pharmacol. Exp. Ther. 254, 711-719.

Vandecasteele, M., Deniau, J.M., Venance, L., 2011. Spike frequency adaptation is developmentally regulated in substantia nigra pars compacta dopaminergic neurons. Neuroscience 192, 1-10.

Whimbey. A,E,, Denenberg, V.H. 1967. Two independent behavioral dimensions in open-field performance. J. Comp. Physiol. Psychol. 63, 500-504.

Yang, E.J., Jiang, J.H., Lee, S.M., Yang, S.C., Hwang, H.S., Lee, M.S., Choi, S.M., 2010. Bee venom attenuates neuroinflammatory events and extends survival in amyotrophic lateral sclerosis models. J. Neuroinflamm. 7, 69-81.

Yang, E.J., Kim, S.H., Yang, S.C., Lee, S.M., Choi, S.M., 2011. Melittin restores proteasome function in an animal model of ALS. J. Neuroinflamm. 8, 69-78.

Zuo, J., Liu, Z., Ouyang, X., Liu, H., Hao, Y., Xu, L., Lu, X.H., 2008. Distinct neurobehavioral consequences of prenatal exposure to sulpiride (SUL) and risperidone (RIS) in rats. Prog. Neuro-psychoph. 32, 387-397.